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THE UNIVERSITY OF ALBERTA

SYSTEMIC EFFECTS OF INCREASED FLUORIDE INTAKE IN CHINCHILLAS AND RABBITS

by

DONALD E.H. SMITH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled SYSTEMIC EFFECTS OF INCREASED FLUORIDE INTAKE ON CHINCHILLAS AND RABBITS submitted by DONALD E.H. SMITH in partial fulfilment of the requirements for the degree of Master of Science in Laboratory Animal Science.

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ABSTRACT

The acute and sub-clinical effects of soluble fluoride administration were studied in the Chinchillas and the Polish Rabbits. Appropriate, pre-calculated quantities of Hydrofluosilicic Acid were added to the experimental animals' drinking water at levels ranging from 0 ppm to 40 ppm fluoride. Elevated levels of fluoride administered resulted in an increase in water consumption. This increase in water consumption appeared to exhibit seasonal fluctuations. No variations in total body weight or weight gain were observed in chinchillas as a result of the experimental fluoride administration.

Fluoride excretion in chinchillas was independent of total fluoride intake, resulting in a positive fluoride balance.

No apparent variations in the hematocrits (packed cell volume) or in the tissue fluoride levels of rabbits or chinchillas exposed to the increased fluoride intake were observed.

The mandibular teeth of chinchillas displayed both increased incisor length and decreased mandibular eruption angles as a result of increased fluoride intake. Increased molar and pre-molar malocclusions were seen in the rabbits and chinchillas exposed to the elevated fluoride levels of drinking water. No variations in tooth pigmentation resulted from the fluoride exposure.

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LITERATURE REVIEW

I. Introduction

Eagers (1969) defined fluorosis or fluoride poisoning as "the physical results of bodily intake of fluorine compounds". Three general types have been described by Eagers. Endemic fluorosis refers to the poisoning effect arising from fluoride intake from natural conditions. Acute fluorosis refers to that type of poisoning characterized by a sudden onset of physiological symptoms produced by a single large dose or quantity of fluoride. Chronic fluorosis refers to that condition arising from a prolonged or repeated administration of fluoride (usually in smaller doses).

II. Chronic Fluorosis as a Recognized Ailment

Although chronic fluorosis has been implicated in many disease syndromes in man, such as allergy (Waldbott, 1956; 1957; 1959), cancer (Peters and Wilke, 1972; Schorr et al., 1972) and neurological complications (Koketsu and Gerand, 1956), the most problematic symptoms of this ailment fall into the category of non-fatal, systemic abberations. In humans, fluoride produces visible effects on tooth enamel known as "Mottled Teeth" (Eager, 1901; McClure, 1962; McClure, 1970), while the skeletal system (Lipkins et al., 1959; Zipkin et al., 1959; Largent, 1961), the thyroid gland (Auskaps and Shaw, 1955; Crawford, 1972), and the kidneys (Smith, 1955; Ramseyer et al., 1957; Singh, 1964) also appear to be affected.

Since the early 1800's, areas with endemic high fluoride levels have had human populations suffering from Denti-Di-Chiae Teeth (Eager,

1901), Colorado Brown-Stained Teeth (Dean, 1933) or Mottled Teeth. McClure (1962) defined these tooth abnormalities as "endemic hypoplasia of the permanent teeth, produced by the ingestion of toxic quantities of fluoride in drinking water". Dean et al. (1936) described this condition when he compared the smooth, glossy, translucent creamy-white color of normally calcified teeth to the dull, chalky-white to brown colored, pitted, mottled teeth. In man, this acquired deterioration was found to be most prevalent among individuals exposed to endemic fluoride in the first eight years of life (Dean et al., 1936). Simons (1965) stated that teeth that were already calcified showed little or no effects of fluoride treatment, while teeth calcified during fluoride exposure showed noticeable signs of mottling. As early as 1901 (Eager, 1901), the prevalence of mottled caries. Because of this relationship, proposals for the prophalactic addition of fluoride to public water supplies were made as early as 1939 by a biochemist, C.J. Cox. As a result, several cities in Wisconsin proceeded with fluoridation schemes before controlled experiments designed to examine chronic fluorosis were initiated. Research reports began to appear in the literature in the early 1950's, but much of this work was based on evidence from localities with endemic high fluoride levels (hard water)--not from samples of defined chemical fluoride additions. Skeletal abnormalities caused by excessive fluoride intake have been reported (Shupe et al., 1962; Shupe, 1966), although few earlier researchers believed that this situation posed any serious threat to public health (McCauley and McClure, 1954; Leone et al., 1954a). Renal damage was postulated by several researchers (Singh, 1964;

Ramseyer et al., 1957) but renal failure as a result of fluorosis has never been reported. Because of the lack of concrete scientific evidence proving severe toxic effects of fluorosis, many municipalities were reassured of the safety of fluoride additions. This assurance resulted in nearly 4000 American communities supplying 82 million people (52.8 percent of the population) with fluoridated water by 1967 (U.S. Department of Health, Education and Welfare, 1968). The same trend has been observed in Canada and has resulted in the fluoridation of local Edmonton water supplies since September, 1967.

III. Endemic Sources of Fluoride

Fluoride has an extremely wide range of appearance in nature. It occurs most abundantly as the mineral fluorspar (CaF_2) and as cryolite ($AlF_3 \cdot 3NaF$), but does appear in small amounts in compounds such as amblygonite [$Li(Al,F)PO_4$], apatite [$Ca_5(Cl,F)(PO_4)_3$] and topaz [$Al_2SiO_4(F \cdot OH)_2$] or as a contaminant in compounds such as muscovite [$KAlSi_3O_{10}(OH)_2$] and limestone ($CaCO_3$). Fluoride usually occurs in surface moving waters at a level less than 1 ppm (Largent, 1961). The transfer of fluoride from soil and water to plants appears minimal. Fluoride is toxic to most plant tissues and only trace levels of fluorides in cereals and cereal products was found by McClure (1949). McClure (1933) did observe that the leaves of these plants contained higher levels than did the stem.

IV. Fluoride Absorption

Using dogs, Carlson et al. (1960) demonstrated that fluoride appeared in the bloodstream as early as 10 min after ingestion, with maximum levels occurring after 60 min. This is due to the fact that

absorption of fluorides occurs in both the stomach and intestine. Stookey et al. (1964a demonstrated that the absorption of fluorides by rats involved a passive process since neither the stomach nor the intestine possessed a system capable of active transport. It should be noted that only the fluoride ion is important in fluoride metabolism. McClure et al. (1945) demonstrated that solubility of 'fluorine supplements' fed to man influenced the amount of fluoride absorbed by the gastro-intestinal tract. It was found that insoluble compounds such as calcium fluoride and cryolite released fluoride ion (via absorption) to the blood stream far slower than soluble compounds such as sodium fluoride or sodium fluosilicate. Using human experimental subjects, Largent (1960) found that 86-97 percent of soluble fluoride in drinking water was absorbed by the gut, while fluorides found in food were as low as 30 percent absorbed. Hodge (1961) contended that elements of the human diet could form complexes with fluorides which could reduce the amount of fluoride absorbed. Stookey et al. (1964a observed in studies on rats that the rate of diffusion of fluoride across the gut wall varied with the age of the rat. Stookey et al. (1964_b) contended that up to 90 percent of the fluoride ingested by rats could become absorbed, but this varied with alimentary time and solubility of fluoride. They further contended that starvation of rats prior to experimental trials resulted in absorbances of fluoride that were increased by as much as 30 percent. Stookey et al. (1964_b) also disputed Hodge's (1961) publication by stating that even though calcium administration decreased fluoride absorption (by precipitation as CaF_2) in rats, phosphate administration increased fluoride absorption. They noted that when calcium and

phosphate were ingested simultaneously by rats, absorption of fluoride was reduced by more than that of calcium alone. Suttie and Phillips (1959) demonstrated that high fat diets fed to cattle increased the toxic effects of fluoride. Suttie and Phillips (1959) stated that, in general, fluorosis was found to be enhanced by poor nutrition, high-fat intake and calcium and vitamin deficiencies. Largent (1961) noted that human fluoride intake increased during the hot summer months, as water intake increased, but fluoride recovered (in urine) was decreased, possibly due to evaporation and apparent increased storage in the body.

V. Fluoride Distribution

Fluoride distribution is thought to follow a simple pattern--fluoride ingested either becomes absorbed by the host or is excreted. Once the fluoride has been absorbed and appears in the bloodstream, it can return to the intestine (a reversal of the passive absorption) to be excreted in feces, it can become collected in the kidney and excreted in urine or can become deposited in body tissue. The greatest limitations of excretion of fluoride is observed by the excretory capacity of the kidney; beyond this capacity, an increase in fluoride intake results in temporary or permanent storage of fluoride in the soft tissues or in bone. Carlson et al. (1960) demonstrated that less than 1 percent of body fluoride of dogs was seen in parotid saliva. Largent (1961) contended that, in man, quantitatively significant amounts of fluoride could be lost as sweat. Fluoride loss can also occur as tooth wear (by normal attrition). A World Health Organization Publication (WHO) (1970) quoted Weidman (1962) as saying that since rodent teeth exhibit constant tooth growth, fluoride loss appears high since normal attrition

constantly wears down teeth. Furthermore, Simons (1965) described teeth from domestic animals, exposed to high fluoride levels, as so prone to fracture that they were known as "dagger teeth".

VI. Systemic Effects of Fluoride Treatment

The fact that fluoride affects the skeletal systems is generally recognized. Fluoride deposition has been verified in laboratory animals, domestic animals and man (Zipkin and Scow, 1956; Lipkins et al., 1959; Zipkin et al., 1959) and has been accepted as an indicator of level of fluoride ingestion. Carlson et al. (1960) demonstrated the skeletal retention of F^{18} isotope in dogs. When levels of fluoride ion in blood are low, hydroxyl ions in hydroxyapatite (solubility...6.6 x 10^{-6} moles/100 ml) in calcified tissues may be substituted by fluorine ions according to the following equilibrium relationship:

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- \rightleftharpoons Ca_{10}(PO_4)_6F_2 + 2(OH)^-$$

The resulting compound is fluorapatite (solubility...1 x 10^{-6} moles/100 ml). The World Health Organization (1970) stated that Perdoc (1963) contended that the strength of the hydrogen bonds of hydroxyapitite were increased (leading to a more stable structure) by the substitution of the hydroxyl ion by fluorine. They further cited Newesely (1961) in contending that "the formation of bone whose mineral component has an apatitic structure or structures would not be possible under biological conditions without the presence of small quantities of fluoride".

If levels of fluoride ion in blood are high, the reaction which could occur is:

$$Ca_{10}(PO_4)_6(OH)_2 + 20F^- \rightleftharpoons 10CaF_2 + 6PO_4^{=} + 2(OH)^-$$

This reaction results in the ultimate deposition of the product CaF_2

(solubility...2 x 10^{-5} moles/100 ml). This compound has yet to be found in bone, but it has been observed in human patients after topical fluoride applications on enamel. Zipkin and Scow (1956) demonstrated that fluoride was not deposited uniformly throughout the skeletal system of rats, but was deposited in the bones in levels proportional to the degree of vascularization of the bone. They further contended that more fluoride was deposited in the epiphyseal region than in the diaphyseal region. Bone abnormalities are a common symptom of chronic fluorosis. As early as 1925 (McCollum et al., 1925), diets containing 226 ppm fluoride (administered as sodium fluoride) produced in rats, heavy bones, irregular in nature, with a dull color and hue. Largent (1961) observed radiographic bone changes in humans exposed to 25 mg fluoride daily. Although extreme calcification and increased bone density were general observations, the author reported the presence of exostoses, especially at the attachment of muscles, tendons and interosseous membranes at the extremities. Largent (1961) also noted that the vertebrae, a common point of attack by fluoride, showed altered proportions and measurements on all planes and exhibited frequent fusion. In Panjab, India (Singh, 1964), patients with a generally poor plane of nutrition exposed to 7.5 ppm fluoride from natural water supplies showed striking changes of the vertebral column (especially in the cervical region). Advanced fluorosis was observed as osteosclerosis and osteophytosis of the spine and as a crippling disease exemplified by bent spines or "poker-back". Jackson (1964) observed an unusual bowing of the tibia in patients from Kenhardt, South Africa who lived on a marginally poor diet and were exposed to a water supply with 6.8 ppm natural fluoride. Simons (1965) described

cattle fed diets with fluoride levels as low as 2 ppm with joints so swollen that the animals refused to stand. Steyn (1964) observed children, supplied with natural water containing 3.6 ppm fluoride, who complained of aches and pains in the legs. Upon examination of these children, the author observed shortened and markedly thickened feet and toes, thickened ankles, and various osteophytes which he attributed solely to their fluoride exposure. In human subjects exposed to natural fluoride levels of 8 ppm in drinking water, Leone et al. (1954b) observed roentographic bone conditions including increased bone density, increased thickening of cortical bone and periosteum, and a general narrowing of the bone marrow spaces. In general, a wide variety of bone abnormalities have been observed, but high fluoride levels, marginal to poor diets, long periods of time and "susceptible" subjects are normally required to produce them.

A recent development has stimulated great interest in the field of cardiac research. Takamori (1957; 1964) observed a greatly increased incidence of myocardial damage among rats exposed to sodium fluoride added to feed. The author demonstrated that rats, fed in excess of 5.84 mg/kg fluoride daily, exhibited myocardial damage directly proportional to fluoride intake. Gross examination of these animals revealed dilated hearts with pale and flabby myocardium, while microscopic examination revealed a regressive degeneration of myocardial fibers, some replacement of lost myocardial fibres with young scar tissue, numerous small hemorrhages and a thickening of vessel walls. Simons (1965) reported that Okushi (1954) observed a higher incidence of myocardial damage in children from a mottled teeth area. However, Simons (1965) did state

that according to health department vital statistics (Illinois State Department of Public Health, 1952), communities with fluoridated water showed no greater risk of death from heart disease than areas without fluoridation schemes.

In general, no major changes appear in the clinical blood assays performed on laboratory animals exposed to high levels of fluoride. McClure and Kornlav (1947) found no variations from the normal in hemoglobin and hematocrit (packed cell volume) readings taken from rats supplied with 50 ppm (added as sodium fluoride) by means of the water supply. Alternatively, Leone et al. (1954a) found statistically significant variations in the white blood count, the lymphocytes and the neutrophils of blood samples taken from human patients drinking water from a reservoir containing water with natural fluoride levels of 8 ppm. Auskaps and Shaw (1955) observed a temporary decrease in hemoglobin in rats given fluoride at levels of 20 ppm via drinking water. Hamamoto (1957), however, observed no significant variations in any parameters of blood assays performed on inhabitants from a mottled teeth area in north central Japan.

Because of the supposed active role that the kidney plays in the excretion of excess fluoride (Simons, 1965; W.H.O., 1970), the possibility of renal damage from animals suffering from chronic fluorosis has been postulated by several workers (Smith et al., 1955; Ramseyer et al., 1957; Singh, 1964). Since both excretion and retention of fluoride is controlled by the kidney, high efficiency of renal clearance is essential. Workers (Largent, 1961; Simons, 1965; W.H.O., 1970) have observed injury (flattening and necrosis) of the columnar cells lining the proximal

convoluting tubules, dilation of Henle's loop and dilation of the distal convoluting tubules (probably due to the blockage of Henle's loop) as a result of elevated fluoride intake. Singh (1964) observed a subtle disturbance of human renal function when patients were exposed to an average of 7.5 ppm fluoride from natural water supplies. Ramseyer et al. (1957) observed histogical changes (interstitual nephritis) that progressively increased as levels of sodium fluoride administered to rats were increased in excess of 50 ppm via the drinking water. The authors also observed renal tubule hypertrophy and hyperplasia. Simons (1965) observed kidney damage in numerous laboratory animals as a direct result of fluorosis, but added that the kidney injury or disease, short of renal failure, did not decrease fluoride excretion via the urine. Smith et al. (1955) induced a high grade tubular injury in rabbits by exposing them to uranium but observed normal fluoride balance, that is, experimental models neither retained nor excreted abnormal proportions of ingested fluoride. It is, therefore, believed that a healthy individual could cope with any renal changes that occurred as a result of chronic fluorosis.

The thyroid gland has been implicated as a major target of attack by the fluoride ion. Simons (1965) postulated several theories of action. One contention was that if the thyroid concentrated fluoride as it does iodide, excessive amounts of fluoride could interfere with thyroid activity by poisoning enzymes. Baumen and Metzger (1949) did postulate that the thyroid had an affinity for all the halides but did not consider the poisonous effects of these materials. It is possible that fluoride could reduce production of thyroid stimulating hormone, which could result in a depression of thyroid activity. Fluoride could

also replace iodide to form fluoridated tyrosine which could result in depressed thyroxine activity and a resultant depression of growth. Although Gordenoff and Minder (in W.H.O, 1970) could not establish a conversion of di-iodotyrosine to mono-iodomonofluorotyrosine, they did produce a partial deiodination of di-iodotyrosine to mono-iodotyrosine by the action of fluoride. They also observed a reduction of activity of the thyroid hormone under the influence of fluoride. The most popular theory, however, is that fluoride competes with iodide causing decreased production of thyroxine and increased incidence of goitre. Crawford (1972) cited several authors when he stated that even moderate levels of fluoride in drinking water could block iodide absorption by the thyroid. She also observed that iodide levels were proportionately lower in soft water than hard water. Auskaps and Shaw (1955) observed a variation in size of the thyroid gland of rats as a result of fluoride administrated via water supplies (20 ppm fluoride). Day and Powell-Jackson (1972) correlated the prevalence of human goitre with fluoride level and hardness of water. They also believed that much of the evidence pertaining to the thyroid gland was contradictory since high iodide intake probably offset any goitrogenic effect that fluoride might have.

Auskaps and Shaw (1955) observed no large variations from normal in rate of growth or final body weight of rats fed eleven months on a diet containing 20 ppm fluoride. Briggs and Phillips (1952) observed a slight weight loss (due to anorexia) in rabbits fed a diet containing 0.0227 percent fluoride. Messer et al. (1973) observed a decreased growth rate in mice fed diets containing as high as 200 ppm fluoride, but attributed this weight loss to a lowered food consumption.

VII. Loss of Tooth Pigment and Tooth Quality as a Result of Fluoride Treatment

Mottled teeth appears in man, in many laboratory animals, and in domestic animals (Dean, 1934; 1936; Newell and Schmidt, 1958; Simons, 1965) as a form of dental dystrophy characterized by dull, chalky-white to brown colored pitted teeth (Dean, 1936). McCollum et al. (1925), however, reported that laboratory rats fed fluoride at levels of 226 ppm (given via the feed) had incisors devoid of the normal orange pigment. Sebrell et al. (1933) stated that mottled teeth in rats (obtained by the administration of 640 ppm fluoride via the diet) appeared as a loss of incisor pigmentation resulting in white incisors with brown striations. Dean et al. (1934) produced the same type of mottled incisors in rats by treating laboratory rats' water supplies with 25 ppm sodium fluoride. McClure (1941) demonstrated that rats with mottled teeth (produced by the administration of 10 ppm fluoride in drinking water) possessed a greater resistance to dental caries than rats given unfluoridated water.

VIII. Fluoride and Tooth Malocclusion

Incidence of malocclusion (or the improper closure of teeth) in laboratory animals is not uncommon. Hill (1967) observed a type II malocclusion (overgrowth of the mandibular incisors) and premolar malocclusion in cottontail rabbits. Weisbroth and Ehrman (1967) observed a type II malocclusion prevalent in several rabbits in their rabbit colony. A severe type II and type III (overgrowth of maxillary incisors) malocclusion exists in several strains of the Bioscience Animal Services rabbit colony at The University of Alberta. Plate I demonstrates the normal occlusion expected from healthy rabbits. Plate II dramatically

Plate I. Normal Occluded Teeth from Polish Rabbit

Plate II. Type II and III Malocclusion in San Juan Rabbit

demonstrates the severity of both type II and type III malocclusion that existed within the University of Alberta rabbit colony. The extreme overgrowth (increased length) of both maxillary and mandibular incisors becomes very evident in these photographs. Note the extreme ulceration of the upper gum as a result of the long mandibular incisors. McCollum et al. (1925) demonstrated that a type II malocclusion (similar to that described above) could be induced in rats by administration of 226 ppm fluoride to the animals via the feed. The authors contended that the overgrowth of the maxillary incisors had been caused by some stimulative action of the fluoride on the osteoblasts, while the mandibular incisors became seriously eroded by contact from the maxillary incisors. The malocclusion became so pronounced that the rats' upper incisors grew backwards into a circle, resulting in ulcerated lesions within the buccal cavity.

Since the incisors can be presented for viewing by the separation of the research animals lips, the variations in the size and shape of each incisor can easily be observed. Since the dimensions of the other types of teeth are not as easily obtained, little or no information is available in the literature regarding molar malocclusions in experimental animals. Discussions with W.M. Samuels (Department of Zoology, University of Alberta) and J.J. Thomsen (Department of Bioscience Animal Services, University of Alberta) suggested that fluoride was suspect as a causative agent of a molar and pre-molar malocclusion in the University pika colony. Furthermore, excess calcium deposition in the molars of children exposed to 5.8 ppm fluoride via drinking water was described by Forrest (1956). Forrest stated that he found "well-formed rounded cusps and shallow

fissures" in the molars. Wallenius (1957) observed an increased width of molars in children supplied with water containing 0.5 ppm fluoride. From the research available, it appears that soluble fluoride administration may produce variations in the size and shape of both incisors and "cheek" teeth.

STATEMENT OF THE PROBLEM

This thesis was designed to demonstrate what effects the addition of excessive amounts of soluble fluoride to drinking water would have on teeth, bones, kidney, liver, blood cells and body weight in chinchillas and rabbits. Because of the problems of incisor malocclusion experienced in the University of Alberta rabbit and pika colony and since the literature suggests that this type of malocclusion could be induced in susceptible laboratory animals exposed to low levels of fluoride intake, the question of whether or not an incisor malocclusion could be induced in Polish Rabbits and Chinchillas exposed to fluoridated waters was posed. In addition, parameters such as calcium, phosphorus, bone strength and body weight were examined in order to reveal any more subtle changes which would essentially be subclinical.

Therefore, the purpose of this study is:

1) To study fluoride intake, excretion and retention in chinchillas to observe any abnormal variations in fluoride metabolism;
2) To study the teeth of Polish Rabbits and Chinchillas to observe any indication of fluoride induced malocclusion; and
3) To examine the experimental animals for subclinical abnormalities as a result of controlled exposures to fluorides.

MATERIALS AND METHODS

I. Introduction

This thesis presents the results of experimentation carried out within the facilities of the Department of Bioscience Animal Services located in the Biological Sciences Building of the University of Alberta. For this research, two species of animals were utilized--the Chinchilla (Chinchilla laniger) and the Rabbit (Oryctolagus cuniculus) var. Polish. The research animals were housed and maintained under conditions surpassing those stipulated by the National Research Council Directives, the Canadian Council of Animal Care's "Care of Experimental Animals: A Guide for Canada", and the Universities Act of the Province of Alberta.

II. Experimental Animals

A. Chinchillas

Since the chinchilla colony (originally containing a population of approximately forty animals) was obtained from a local chinchilla breeder, precise standardization of chinchillas (similar sex, age and weight) was not feasible. On July 1, 1973, sixteen chinchillas (chosen because of the same sex, approximate age and approximate weight) were randomly divided into four experimental groups. Each group of four chinchillas was tattooed, weighed, processed (provided with cage cards containing pertinent data) and supplied with water containing 0, 1, 10 or 20 parts per million (ppm) fluoride. In order to observe any variations in the effects of high fluoride administration upon younger animals, five pregnant females and two young chinchillas (weaned from a gravid

female that was supplied with water containing 20 ppm) were supplied with water containing 40 ppm fluoride, November 1, 1973.

B. Rabbits

Due to difficulty in production of offspring of the chinchillas (difficulty in breeding and a long gestation period of 119 days), it was decided that further experimentation on the effects of excessive fluoride intake on younger animals could best be achieved by direct experimentation upon rabbits. On November 1, 1973, six Polish Rabbit does and two Polish Rabbit bucks were obtained by the department for experimental purposes; the does were divided into three experimental groups and supplied with water containing fluoride levels of one of 1 ppm, 20 ppm and 40 ppm, processed and bred. Young rabbits obtained from these breedings were kept when weaned and maintained on the same water treatment as their mother and observed until the conclusion of the experiment.

III. Housing

A. Chinchillas

Experimental chinchillas were kept in environmentally controlled quarters under regulated light (12 hr light and 12 hr dark), controlled humidity (57% $\pm$ 1.0% relative humidity) and controlled temperature (66°F $\pm$ 1°F). Controls (relative humidity--Johnson Service Co. and temperature--International Register Co.) used in the maintenance of these rigid environmental conditions are illustrated in Plates III and IV. The high relative humidity of the chinchilla room was maintained to prevent respiratory complications resulting from low humidity levels. The cool temperature of the chinchilla room was maintained at a its level, as suggested by the supervisor of the Bioscience Animal Center, to allow

Plate III. Temperature and Humidity Controls for Chinchilla and Rabbit Rooms

Plate IV. Diurnal Light Control for Chinchilla and Rabbit Rooms

for adequate comfort to the chinchilla. Each animal was individually housed in a metal cage (31.8 cm x 35.6 cm x 55.9 cm) that was supported on a moveable rack of eight cage capacity (Plate V). Experimental chinchillas were checked daily by qualified animal technicians to ensure that each animal was healthy and had an adequate supply of food and water. Fresh bedding and clean cages were supplied to the animals once every second week to ensure maximum comfort to the research animals. The room, measuring 4.17 m x 3.22 m x 2.68 m, contained only experimental chinchillas used in this project. The floor of the room housing the chinchillas was swept daily and washed at least once a week.

B. Rabbits

Experimental rabbits were kept in environmentally controlled quarters under regulated light cycle (12 hr light and 12 hr dark), controlled humidity (54.0% ± 1.0% relative humidity) and controlled temperature (69°F ± 1°F). The high relative humidity of the rabbit room was maintained to prevent respiratory complications (especially snuffles) resulting from low humidity levels. The cool temperature of the rabbit room was maintained at its level, as suggested by the supervisor of the Bioscience Animal Center, to allow for adequate comfort to the rabbit. Each rabbit was individually housed in a cage of wire construction (33.6 cm x 41.7 cm x 60.9 cm) that was supported on a moveable rack of six cage capacity (Plate VI). Experimental rabbits were checked daily by qualified animal technicians to ensure that each animal was healthy and had an adequate supply of food and water. Pans of absorbant material (for collection of excrement) were replaced twice weekly and rabbit cages were washed weekly. The room, measuring 3.34 m x 3.04 m x 2.85 m,

Plate V. Chinchilla Cages on Moveable Rack

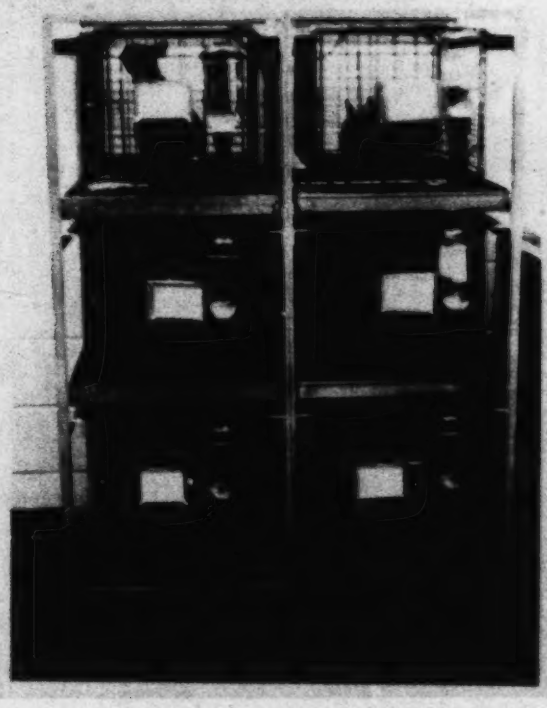

Plate VI. Rabbit Cages on Moveable Rack

contained approximately 20 other non-experimental Polish Rabbits. The floor of the room housing the rabbits was swept daily and washed at least once a week.

IV. Experimental Diets

A. Feed

Each experimental rabbit was supplied daily with feed, measured from a cup containing approximately 100 g of Master Feeds Baby Rabbit Pellets (analysis of feed found in Table I). Master Feeds Baby Rabbit Pellets were fed to the chinchillas (because of similarity in analysis of the two diets). Since the chinchilla wastes a large portion of the feed given it (the animals tend to "play" with the feed), experimental chinchillas were fed ad libitum (food consumption was calculated by metabolic trials to be approximately 10 to 15 g daily). Random samples of the commercial Baby Rabbit Pellets were sent to the Research Council of Alberta for fluoride analysis to aid to establish the basal level of fluoride intake via the feed. The results of this analysis are found in Table II.

B. Water

Levels of fluoride found in municipal water supplies were monitored daily (Table III) during August, 1973. Thereafter, weekly samples were taken, until November 30, 1973, to determine the constancy of fluoride levels in the local reservoir. Once these values were recorded, levels of natural occurring fluorides (levels of fluoride in water before fluoride addition) were obtained from the records of the Water and Sanitation Department of the City of Edmonton.

TABLE I. Elemental Analysis of Master Feed Baby Rabbit Pellets

Crude Protein (minimum)	18.0%
Crude Fat (minimum)	2.5%
Crude Fiber (maximum)	12.0%
Salt	0.5%
Calcium	1.3%
Phosphorus	0.75%
Vitamin A (minimum international units/lb)	4000, 5000 (including carotene)

TABLE II. Fluoride Analysis of Master Feed Baby Rabbit Pellets

Sample	ppm F Wet	ppm F Dry	% Moisture
I	24.8	27.8	10.8
II	25.6	28.7	10.9

TABLE III. Natural Fluoride Levels and Total Fluoride Levels of City of Edmonton Water Supplies from August 1, 1973 until November 14, 1973

Date	Natural Fluoride[1] Level of Water (ppm)	Total Fluoride[1] Levels of Water (ppm)	Total Fluoride Levels of Water as Determined by Experimentation (ppm)
Aug 1	.08	1.00	1.00
2	.08	1.00	.90
3	.09	1.00	.95
4	.11	1.00	.99
5	.08	.99	.95
6	.11		1.00
7	.08	1.02	.95
8		1.00	.95
9		1.02	1.00
10	.15	.98	1.05
11	.12	.12	
12	.11	..07	
13	.13	1.02	.97
14	.14	1.00	.99
15		1.00	.95
16		1.05	
17	.14		.95
18	.17	1.00	.99
19	.07		.95
20	.14	1.00	.96
21	.11	1.00	.95
22		1.00	.95
23		1.00	.95
24	.15	.97	.94
25			.96
26			.95
27	.10	.95	.97
28	.14	.91	.98
29		.98	.95
30		.90	
31	.15	1.02	.99
Sept 1	.10	1.00	1.00
10	.14	.93	.94
17	.04	.90	.90
24	.11	.99	1.00
30	.04	.99	1.00
Oct 5	.12	.89	.89
12	.08	1.00	1.00
19	.08	.92	.93
26	.00	.98	1.00
Nov 2	.10	1.00	1.00
9	.06	1.00	1.00
16	.14	1.00	1.00

[1]Values obtained courtesy of the City of Edmonton, Water & Sanitation Dept.

Experimental animals were supplied with water containing levels of fluoride consistent with the experimental grouping. The experimental fluoride groups were as follows:

Group I Rabbits and Chinchillas - water containing 20 ppm fluoride

Group II Chinchillas - water containing 10 ppm fluoride

Group III Rabbits and Chinchillas - water supplied from "city water supplies" or "tap water"

Group IV Chinchillas - water containing 0 ppm fluoride [double distilled (de-ionized) water]

Group V Rabbits and Chinchillas - water containing 40 ppm fluoride

Water intake was monitored and recorded daily for each experimental animal.

Fluoridated waters for this experiment were prepared by the addition of appropriate, pre-calculated quantities of commercial 30% Hydrofluosilicic Acid (the chemical used by the City of Edmonton for its fluoridation of local water supplies) to 20 liter volumes of distilled water.

V. Metabolic Trials on Chinchillas

Metabolic trials were carried out to quantitatively illustrate absorption, excretion and retention of fluoride by the chinchilla.

Two chinchillas from each of Group I, II, IV and V were placed in specially modified mouse metabolism cages for a period of three days (two days prior to trial to allow experimental animals to become accustomed to the new cage and one day of experimental trial).

A. Metabolism Cages

Econo metabolism units (Maryland Plastics Inc.), 20.2 cm in diameter, were supplied with a wire (1/2" x 1/2" mesh) extension unit, 25.6 cm in height, to allow more comfort for the experimental chinchilla. Specially designed feeders, made of galvanized metal, were prepared to allow feed to be accurately administered to the experimental animal. Water bottles supplied with the metabolic cages proved adequate for the experimental trials. Plate VII illustrates the assembled metabolic unit.

B. Feed

Each animal was fed 25 g of Master Feed Baby Rabbit Pellets. After twenty-four hours had elapsed, the unconsumed food was removed from the feeders and from the feces collection container (food "played" with by the animals), weighed and the amount of food consumed, as determined by difference, was recorded.

C. Water

Each animal was given 125 ml of its respective group's water. Care was taken to place the water bottles such that water could not be wasted. After twenty-four hours had elapsed, the unconsumed water was measured and the amount of water consumed, as determined by difference, was recorded.

D. Feces

After twenty-four hours had elapsed, feces were collected from the feces container of the metabolism cage, weighed and placed in 20 ml glass sample bottles and refrigerated at 11°C. After samples from one complete trial were obtained, samples were removed from refrigeration and sent to the Research Council of Alberta for fluoride analysis.

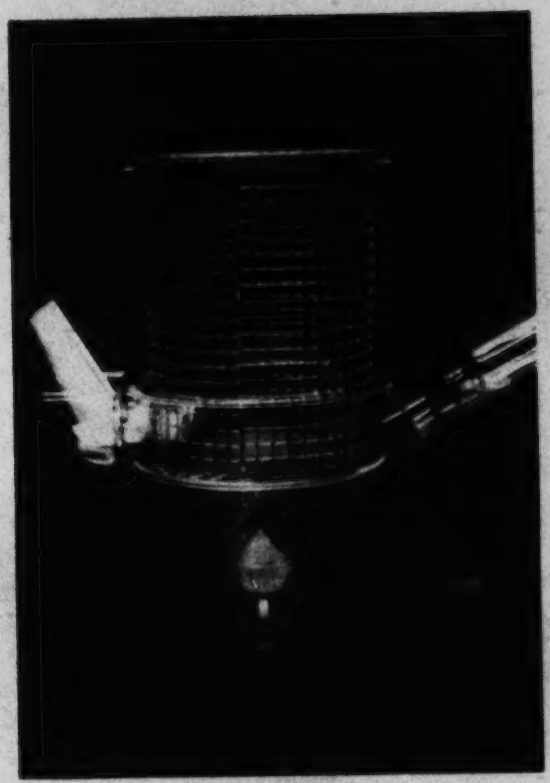

Plate VII. Metabolic Cage
Note: Water Bottle and Holder on right of cage, feeder on left of cage, wire extender (to allow more comfort to animals), glass bulb on bottom of cage to allow urine to drip into the urine cup (immediately below bulb) and feces collection container (urine cup supported within this container).

E. Urine

After twenty-four hours had elapsed, urine was collected from the urine cup of the metabolism cage, measured (in ml), prepared with Perchloric Acid and Silver Perchlorate (to remove chloride ion) using the method described by Derner (1966), placed in 20 ml glass sample bottles and refrigerated at 11°C. After samples from one complete trial were obtained, samples were removed from refrigeration and sent to the Research Council of Alberta for fluoride analysis.

VI. Clinical Blood Determinations

A. Hematocrit

To verify the findings of McClure and Kornlay (1947), hematocrits (packed cell volume) were performed on all chinchillas and rabbits. In each species, ears of the experimental animals were sterilized with ethanol, nicked in the marginal ear vein with a razor blade, and samples removed and collected in Fischer Scientific Heparinized Hematocrit Capillary Tubes and centrifuged for 5 min on a model MB Micro-Hematocrit Centrifuge (International Equipment Co.). Samples were then placed in a Micro-Capillary Reader (International Equipment Co.) and values recorded.

B. Complete Blood Count

Four rabbits (one Group III rabbit, two Group II rabbits and one Group V rabbit), chosen because of their abnormal hematocrit values, were tested for further abnormal blood findings. To do so, each animal was manually restrained and the technique of cardiac puncture (using a 21-gauge needle) was used to obtain 5 ml samples of blood. Blood was then placed in a sealed glass test tube containing EDTA, taking care to leave one drop of blood so that a blood smear could be made, and sent to Dr. S. Hanson & Associates (Edmonton) for analysis.

VII. Animal Termination

Each experimental animal was weighed and weight and weight gain (as determined by difference of initial weight and final weight) recorded. Since part of the rabbit and chinchilla colony was to be utilized in experimentation outside of this thesis project, not all research animals were terminated. Animals to be terminated were humanely terminated by intercardial injections of Euthanal (concentrated lethal solution of Nembutal). Post-mortems were performed to observe any visible systemic abberations. Liver and kidney samples from eight rabbits (four samples from four Group I rabbits, three samples from three Group V rabbits and one sample from one Group IV rabbit) were kept, frozen and sent to the Veterinary Services Division of the Alberta Department of Agriculture for fluoride analysis. From each experimental animal (Chinchilla and Rabbit) the skull and long bones were removed, cleaned (excess skin and muscle removed) and frozen for further testing.

VIII. Bone Studies

A. Bone Strength

Since Largent (1961) and Singh (1964) observed radical alterations in bone density as a result of fluoride treatment, tests were undertaken to observe any variations that might occur in bone strength (as measured by force required to fracture bones). For this set of tests, stored bones were removed from the freezer 24 hr prior to testing. Circumference of humerus 6 mm from head of humerus was measured and marked with a felt pen on each sample. Bones were then severed using a Model MSl-12 Sheer Strength Tester (Bodine Electrical Co.). The force required to break the bones per unit of circumference was then recorded.

B. Bone Calcium

Random bone samples were removed from the freezer 24 hr prior to testing, ground using a Model 700S Blender (Waring Co.) and placed in 1 g portions in porcelain crucibles. Samples were charred and placed in a 800°C muffler furnace for 4 hr for ashing. Samples were then tested for levels of calcium using the method described by Moss (1961).

C. Bone Phosphorus

Random bone samples were removed from the freezer 24 hr prior to testing, ground using a Model 700S Blender (Waring Co.) and placed in 1 g portions in porcelain crucibles. Samples were then treated with 5 ml of a magnesium nitrate solution, a few grains of magnesium oxide powder and allowed to sit overnight. The next day, samples were boiled dry on a hot plate and placed in a 1200°C muffler furnace for 1 hr. Samples were then tested for levels of phosphorus using the method described by Donald et al. (1956).

D. Skulls

1. Preservation

Frozen skulls were placed in 1000 ml beakers containing 5-10 ml of commercial dishwashing liquid and 500 ml of water. Beakers, containing the skulls in the soap-water solution, were then boiled over a bunsen burner for 2 to 3 hr. Skulls were removed from the beaker and all remaining muscle and tissue removed.

2. Tooth Parameters

Preserved skulls were separated into mandibular jaw and skull and maxillary jaw, wrapped in cotton batting and placed in plastic storage containers until utilized.

a. Incisor Lengths

Length of incisor was measured for each of the experimental animal's incisors. Length of incisor was obtained by taking a thin string over the length of incisor to be measured, marking the end of incisor by a mark on the string and measuring length of string marked with dial calipers. Incisor length was then recorded. To standardize these values, diameter of skull was measured (using the same techniques as above) and recorded. Incisor length was then considered in terms of length of incisor per centimeter of skull diameter.

b. Tooth Eruption

A moveable cardboard L-square was designed and made to pictorally demonstrate the angle of tooth eruption to be observed. Once the angle of eruption was transferred to paper, the angle was measured using a protractor and recorded. Tooth eruption angles were measured, as illustrated by Plates VIII, IX and X, and recorded for each experimental animal.

c. Incisor Gap

The space between each set of incisors was measured in chinchillas and rabbits (using dial calipers).

d. General Observations

Once calculations were made, each jaw was closely examined to observe tooth quality and tooth coloration (pigmentation) and photographs of unusual jaws or teeth were taken.

Plate VIII. Measurement of the Greater Angle of Mandibular Incisor Eruption
Note: This angle indicates how erect the incisors are.

Plate IX. Measurement of the Lesser Angle of Mandibular Incisor Eruption
Note: This angle indicates the amount backward curve of the teeth.

Plate X. Measurement of the Angle of Maxillary Incisor Eruption

OBSERVATIONS

I. Introduction

When reviewing the experimental data, one must consider that fluoride levels in feed remained constant (Table II) while fluoride levels of water varied with each respective group. Standardization of values has occurred, where feasible, by the comparison of values to a fixed unit basis. Since a disease syndrome which could effect several parameters was observed in the rabbit colony, many experimental parameters were reported as data in its respective table but not commented upon under "Observations".

II. Body Weight and Body Weight Gain

A. Chinchillas

Initial body weights and final body weights are listed (Table IV) for most experimental animals. Since younger animals would have lighter weights and higher weight gains than older animals, Group V chinchillas were not included in Table IV. Initial body weights of experimental animals are constantly used in this section as a reference point for standardization of experimental data. From the data obtained on final body weights, one observes that the average final body weight for Group I chinchillas was greater than average final body weights for Group II or Group III chinchillas. Group IV chinchillas (control animals), however, had an average final body weight greater than any of the other groups. Since Group IV chinchillas had a higher average initial body weight than any other experimental group, body weight gain (Table IV) was then considered. In order to standardize values, body weight gain was

TABLE IV. Initial Body Weight, Final Body Weight and Total Weight Gain/Kg Initial Body Weight of Experimental Chinchillas

Animal Group	Animal	Level of Fluoride Administered(ppm) Via Feed	Via Water	Initial Body Weight (grams)	Group Average	Final Body Weight	Group Average	Body Weight Gain	Body Weight Gain/Kg Initial Body Weight	Group Average	Median
I	1	25.2	20	523		640		117	183		
I	2	25.2	20	453	468	468	551	15	32	138	160±74
I	3	25.2	20	478		502		24	48		
I	4	25.2	20	421		592		171	289		
II	1	25.2	10	471		566		95	167.8		
II	2	25.2	10	521	455	460	484	-61	-133	54	18±150
II	3	25.2	10	453		496		43	87		
II	4	25.2	10	375		413		38	92		
III	1	25.2	1	410		410		0	0		
III	2	25.2	1	438	442	439	473	1	2	60	67±67
III	3	25.2	1	476		549		73	133		
III	4	25.2	1	446		495		51	103		
IV	1	25.2	0	432		436		4	9		
IV	2	25.2	0	577	508	638	561	61	96	85	150±154
IV	3	25.2	0	472		619		147	237		
IV	4	25.2	0	553		551		-2	-4		

expressed as body weight gain per kilogram of animals initial weight. Although chinchillas exposed to the control diet (Group IV) had a high weight gain (higher than Group II or III), the weight gain for Group I chinchillas was almost double that of the control diet. The large degree of biological variability was demonstrated by the fact that Group III animals and Group IV animals (only separated in experimental trials by 1 ppm fluoride in the water) showed extreme variations in body weight gain.

B. Rabbits

Several rabbits within the experimental colony became extremely ill (extreme weight loss and lethargic condition of animals) during the experimental trials and were terminated. On April 29, 1974, the Veterinary Services Division of the Alberta Department of Agriculture isolated a protozoan, Nosema cuniculi, as the causative agent of this disease situation. Upon completion of post-mortems on terminated rabbits, physical evidence of 'Nosematosis' was apparent in approximately two-thirds of the dissected animals. For this reason, animal body weights from animals suffering from extreme symptoms of Nosematosis were not included in data tabulation (Table V). Since younger rabbits would have lighter weights and higher weight gains than the adult rabbits, rabbits weaned after January 1, 1974, were not included in this table. Because of the infectious disease syndrome present in the colony and the deviations from the normal that this disease situation could pose, this aspect of the experiment was de-emphasized.

III. Water Consumption

A. Chinchillas

TABLE V. Initial and Final Body Weight for Individual Rabbits[1]

Rabbit	Level of Fluoride Administered (ppm) Via Feed	Via Water	Initial Body Weight (grams)	Final Body Weight (grams)
Group III Animal 1	25.2	1	1530	1955
Group II Animal 2	25.2	10	1460	1643
Group I Animal 1	25.2	20	1435	1623
Group I Animal 3	25.2	20	1480	1640
Group V Animal 1	25.2	40	1385	1890
Group V Animal 2	25.2	40	1410	1620

[1]Weanling rabbits (weaned after Jan. 1/74) placed on treated water and rabbits terminated because of Nosematosis were not included with data because of obvious experimental error.

From the data found in Table VI, one observes that no great variations in water consumption occurred between chinchillas exposed to experimental fluoridated water of Group II, Group III or the unfluoridated water of Group IV. At fluoride levels of 20 ppm (Group I water), water consumption was dramatically increased. This increased average water consumption was consistent for each animal in Group I and was not caused by an excessive intake from one or two animals.

Table VII demonstrates the variations that occurred among chinchilla when considering the average monthly water consumption. By comparing the average water consumption of the four groups of chinchillas for each month, it becomes apparent that the monthly variations in water consumption were constant among the four groups. One also observes the trend that water consumption gradually decreased from October, 1973 until December, 1973 and increased from January, 1974 until April, 1974.

B. Rabbits

Water consumption (Table VIII) for the three experimental rabbit groups appeared to remain relatively constant. Since 'Nosematosis' had been diagnosed within the rabbit colony, the reliability of these values may have been influenced by this disease syndrome.

IV. Metabolic Trials

From the data contained in Table IX, one can observe the fluoride intake from each of water supplies and food supplies and the total fluoride intake of the four experimental chinchilla groups. Fluoride intake was then standardized by transforming these values to a fluoride intake per kilogram body weight basis. From

TABLE VI. Average Monthly Water Consumption (ml)/Kg Average Initial Body Weight for Individual Chinchillas

Group	Level of Fluoride Administered(ppm) Via Feed	Via Water	Average Monthly Water Consumption Animal 1	Animal 2	Animal 3	Animal 4	Group Average	Median
I	25.2	20	3344	2478	2390	2365	2644	2854±465
II	25.2	10	1908	1457	1546	2663	1894	2060±603
III	25.2	1	1927	2541	1821	1384	1918	1962±579
IV	25.2	0	2202	1787	1761	1742	1873	1972±230

TABLE VII. Total Monthly Water Consumption(ml)/Kg Average Initial Body Weight for Specified Chinchilla Groups(fluoride in water)

Month	Group I 20 ppmf Via Water	Group II 10 ppmf Via Water	Group III 1 ppmf Via Water	Group IV 0 ppmf Via Water
September	2250	1488	1860	1569
October	2904	1829	1945	1497
November	1742	1322	1268	856
December	1742	1322	1268	856
January	2788	1653	1522	1902
February	1684	1488	1522	1711
March	2091	1488	1522	1902
April	2323	1818	1945	1854

TABLE VIII. Average Monthly Water Consumption for Individual Rabbits[1] per Kilogram of Final Body Weight

Rabbit	Level of Fluoride Administered(ppm) Via Feed	Via Water	Average Monthly Water Consumption /Kg Body Weight
Group III Animal 1	25.2	1	2634 ml
Group I Animal 1	25.2	20	2763 ml
Group I Animal 3	25.2	20	2784 ml
Group V Animal 1	25.2	40	2696 ml
Group V Animal 2	25.2	40	2364 ml

[1]Weanling rabbits (weaned after Jan. 1/74) placed on treated water and rabbits terminated because of Nosematosis were not included with data because of obvious experimental error.

Table IX, one observes that as fluoride level of water was increased, total fluoride intake was increased. By comparing the fluoride intake (Table IX) of the experimental chinchillas to the urinary fluoride output (Table X), one observes that urinary fluoride output was independent of fluoride intake via the water, fluoride intake via the feed and total fluoride intake. One also observes that even though fluoride levels of water and total fluoride levels were greatly increased, total urinary fluoride and fluoride concentration of urine remained relatively constant. Only Group II Animal II had urine fluoride concentrations significantly higher than the other experimental animals. When comparing the fluoride intake (food, water and total fluoride intake) found in Table IX to the fecal fluoride output found in Table X, it became obvious that fecal fluoride (excretion) was a direct function of fluoride administration via the feed but was independent of both fluoride administration as water and total fluoride intake. By comparing fluoride input via the feed (Table IX) in Group IV Animals (no fluoride administered via the water) to the fluoride excreted in urine and feces (Table X), it appears that approximately one-third of the fluoride administered as feed remained within the body.

Data from Table XI demonstrates the positive fluoride balance (or apparent storage) for each of the experimental animals tested. From this table, one observes that the degree of fluoride balance appears to be a direct function of level of fluoride administered in water. When no fluoride was present in the water, only small amounts of fluoride remained in the body. This fluoride (amount remaining in body) came from the fluoride administered in feed. As fluoride levels were increased

TABLE IX. Fluoride Intake—Comparison of Fluoride Intake via Feed and Water to Total Fluoride Intake and Total Fluoride Intake/Kg of Initial Body Weight in Experimental Chinchillas

Animal	Fluoride Intake (Feed)	Fluoride Intake (Water)	Total Fluoride Intake	Total Fluoride Intake /Kg Body Weight
IV 1	325 µg	0 µg	325 µg	745 µg
IV 5	204 µg	0 µg	284 µg	653 µg
II 2	247 µg	100 µg	347 µg	754 µg
II 3	499 µg	20 µg	519 µg	945 µg
I 5	423 µg	400 µg	823 µg	1837 µg
V 4	339 µg	830 µg	1169 µg	2675 µg
V 7	370 µg	610 µg	980 µg	2350 µg

TABLE X. Total Fluoride Intake/Kg of Initial Body Weight Versus Fecal Fluoride/Kg of Initial Body Weight, Urinary Fluoride/Kg of Initial Body Weight and Concentration of Fluoride in Urine in Experimental Chinchillas

Animal	Total Fluoride Intake/Kg Body Weight	Concentration of Fluoride in Urine	Urinary Fluoride /Kg Body Weight	Fecal Fluoride /Kg Body Weight
IV 1	745 µg	4.63 µgF/ml	133 µg	394 µg
IV 5	653 µg	5.21 µgF/ml	193 µg	255 µg
II 2	754 µg	13.5 µgF/ml	234 µg	0 µg
II 3	945 µg	5.5 µgF/ml	151 µg	801 µg
I 5	1837 µg	3.25 µgF/ml	138 µg	96 µg
V 4	2675 µg	6.19 µgF/ml	192 µg	116 µg
V 7	2350 µg	4.30 µgF/ml	199 µg	145 µg

TABLE XI. Fluoride Balance/Kg Body Weight Versus Fluoride Intake in Experimental Chinchillas

Animal	Level of Fluoride Administered (ppm) Via Feed	Via Water	Total	Fluoride Balance /Kg Body Weight
IV 1	25.2	0	25.2	+218 µg
IV 5	25.2	0	25.2	+221 µg
II 2	25.2	10	35.2	+520 µg
II 3	25.2	10	35.2	+357 µg
I 5	25.2	20	45.2	+714 µg
V 4	25.2	40	65.2	+2082 µg
V 7	25.2	40	65.2	+2258 µg

to 10 ppm in the water, fluoride retained by the host was more than doubled. As water fluoride levels were doubled to 20 ppm, fluoride retention by the host, again almost doubled. As water fluoride levels were increased to 40 ppm, fluoride retention was almost tripled.

V. Clinical Blood Assay

A. Hematocrit

1. Chinchillas

From the data present in Table XII, one observes that even though Group I animals had slightly higher hematocrit levels, no significant variations existed in hematocrit between the five experimental treatments using chinchillas.

2. Rabbits

No statistically valid variations in hematocrit (Table XIII) were observed between the three experimental groups using rabbits.

B. Complete Blood Assay

Since sample size for the blood assays were small and since a large segment of the rabbit experimental group was suffering from Nosematosis, this aspect of the experiment was de-emphasized, but from the data obtained, animals exposed to higher experimental levels of fluoride were found (Table XIV) to have a lowered red blood cell count, a lowered hemoglobin level, fewer neutrophils and more monocytes.

VI. Tissue Fluoride Levels

From the data present in Table XV, only a slight increase in fluoride level of kidney tissue was apparent in rabbits subjected to increased fluoride administration via the water. Wide variations in

TABLE XII. Hematocrit of Chinchillas Exposed to Various Levels of Dietary Fluoride

Group	Level of Fluoride in Water	Animal 1	2	3	4	5	6	7	9	10	Group Average	Median
I	20 ppm	46			47	47			49		47.3	47.5±1.5
II	10 ppm			44	45						44.5	44.5±0.5
III	0 ppm	43	48	50	43						46.0	46.5±3.5
IV	1 ppm	45	47	46	46	45	46				45.8	46.0±1.0
V	40 ppm	44		45	46		50	46	47	46	46.3	47.0±3.0

TABLE XIII. Hematocrit of Polish Rabbits Exposed to Various Levels of Dietary Fluoride

Animal	Level of Fluoride in Water	Hematocrit %	Group Average	Median
III 1	0 ppm	44.7		
IV 1	1 ppm	38.9		
III 4	1 ppm	43.4	42.0	41.8±2.9
IV 1	1 ppm	40.8		
I 1	1 ppm	42.0		
I 4	20 ppm	45.3		
I 5	20 ppm	47.0		
I 6	20 ppm	44.0	43.9	44.0±3.0
I 7	20 ppm	40.9		
I 8	20 ppm	44.0		
V 4	40 ppm	43.0		
V 5	40 ppm	40.0	41.2	41.9±1.1
V 6	40 ppm	40.7		

TABLE XIV. Complete Blood Picture and Differential Count from Polish Rabbits Exposed to Various Levels of Dietary Fluoride

Animal	Level of Fluoride in Water	WBC $x10^3/mm^3$	HGB gm/dl	RBC $x10^6/mm^3$	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
III 1	1 ppm	5.1	13.9	6.13	8	36	6	47	3
I 1	20 ppm	7.3	13.4	6.38	8	30	7	54	1
I 3	20 ppm	2.7	12.2	5.25	7	42	12	38	1
V 6	40 ppm	6.2	12.4	5.66	4	22	9	61	4

TABLE XV. Level of Fluoride in Kidney and Liver in Polish Rabbits Exposed to Various Levels of Dietary Fluoride

Animal	Level of Fluoride Administered (ppm) Via Feed	Via Water	Level of Fluoride in Kidney (ppm) Value	Average	Median	Level of Fluoride in Liver (ppm) Value	Average	Median
III 1	25.2	1	.9	.9	.9	.4	.4	.4
I 1	25.2	20	.9			1.1		
I 3	25.2	20	.9	1.1	1.4±.4	.4	1.3	1.1±.4
I 6	25.2	20	.8			1.5		
I 7	25.2	20	1.81			.8		
V 4	25.2	40	1.2			1.0		
V 5	25.2	40	1.3	1.6	1.7±.5	1.0	.8	.7±.3

fluoride levels of kidney tissue existed within each experimental group. Only Group I Animal 7 and Group V Animal 7 exhibited high fluoride retention levels, while all other rabbits in each respective group retained fluoride in levels equal to or slightly higher than control rabbits.

Wide variations in fluoride content of liver tissue (Table XV) existed among the various experimental groups. In general, fluoride levels in liver tissue were higher for all fluoride treated rabbits than for control rabbits. An interesting feature of Table XV is that those animals with high fluoride levels in kidney tissue had lower liver fluoride levels than those animals with lower kidney fluoride levels.

VII. Bone Strength and Bone Composition

A. Bone Breaking Strength of Chinchillas

Because of the variations in circumference of bones (Table XVI), values pertaining to breaking strength of bones of chinchillas (Table XVI) were standardized to breaking strength of bones per unit of circumference (Table XVI). From data in this table, it was found that chinchillas from Group V had bones that were extremely difficult to break (as demonstrated by amount of physical force required to break them). One also observed that in most cases, as amount of fluoride administered to chinchillas (via water) was increased, so did breaking strength. Bones from chinchillas given Group III water (1 ppm fluoride) had breaking strengths that were greater than those obtained from any other group. This observation of high breaking strength contradicted those values obtained from Group IV animals (0 ppm fluoride) who had very low breaking strength values.

TABLE XVI. Breaking Strength Per Unit of Circumference Versus Fluoride Administered in Chinchillas

Animal	Level of Fluoride in Water (ppm)	Circumference (mm)	Force to Break Bones (M_V)	Breaking Strength/ Unit of Circumference (M_V/mm)	Median
IV 3	0	4.9	341	69.6	64.8±4.8
IV 4	0	4.9	293	60.0	
III 2	1	4.6	385	84.0	85.0±1.1
III 3	1	4.7	405	86.1	
II 2	10	5.5	382	65.6	68.4±2.8
II 3	10	5.5	392	71.1	
I 3	20	4.5	280	62.9	62.9
V 1	40	4.7	361	78.9	
V 4	40	4.6	389	84.9	
V 6	40	4.5	385	85.1	82.3±3.4
V 7	40	4.7	350	83.3	
V 8	40	4.7	372	79.1	

TABLE XVII. Calcium and Phosphorous Studies Versus Fluoride Administered in Chinchillas

Animal	Level of Fluoride in Water (ppm)	Calcium in Bone (MgCa /gm bone sample)	Phosphorous in Bone (MgP/gm bone sample)	Ca/P
IV 1	0	215	81	2.65/1
IV 6	0	185	87	2.13/1
V 6	40	190	90	2.11/1

B. Calcium and Phosphorus Content of Bones of Chinchillas

From the data found in Table XVII, no major variations in bone calcium or bone phosphorus were found in trials performed on chinchillas of the two extreme fluoride treatments (10 ppm and 40 ppm fluoride in drinking water). Although the calcium to phosphorus ratio was slightly lower in the Group V chinchilla bones than in the Group IV chinchilla bones, the value was not low enough to be considered significant.

VIII. Tooth Studies

A. Tooth Length

1. Chinchillas

Although incisor length (Table XVIII) could have been studied as a parameter of fluorosis, incisor length was standardized by comparing length of chinchilla incisors to diameter of skull (as measured by diameter of skull across the orbital sinus). By comparing standardized data found in Table XVIII, it is obvious that no great variations in length of maxillary incisors existed between the five experimental groups. Throughout the experiment, however, certain chinchillas had exhibited consistent extreme variations from the median of animals in its given group. Such an animal was Group II Animal 2 which exhibited maxillary incisors that were far longer than any other research chinchilla.

The standardized length of mandibular incisors (Table XVIII) appears to be a more reliable indicator of fluoride administration via the water. Although animals in the experimental chinchilla groups that were treated with water containing up to 10 ppm fluoride did not appear to be affected, water fluoride administration of levels of 20 ppm

TABLE XVIII. Tooth Measurements Versus Fluoride Treatment in Chinchillas

Animal	Level of Fluoride in Water (ppm)	Diameter of Skull as Measured by Circumference at Orbital sinus (cm)	Length Upper Incisor (mm)	Length Upper Incisor/Unit Skull Dimension (mm/cm)	Length Lower Incisor (mm)	Length Lower Incisor/Unit Skull Dimension (mm/cm)
IV 1	0	3.2	3.0	1.0	4.4	1.4
IV 2	0	3.2	3.7	1.2	4.4	1.4
IV 4	0	3.5	4.4	1.3	4.8	1.4
III 2	1	3.4	3.7	.9	4.2	1.2
III 3	1	3.3	4.0	1.2	4.4	1.3
III 4	1	3.1	3.6	1.7	4.5	1.4
I 3	20	3.3	3.9	1.7	4.3	1.3
I 5	20	3.7	2.9	.8	3.3	.9
II 2	10	3.3	4.9	1.5	5.6	1.7
II 3	10	3.3	3.5	1.0	4.8	1.4
V 3	40	3.4	4.4	1.3	4.9	1.5
V 4	40	3.4	3.4	1.0	4.7	1.4
V 6	40	3.3	3.5	1.1	4.0	1.2
V 7	40	3.0	3.6	1.2	4.0	1.4
V 9	40	3.1	3.2	1.0	4.7	1.5
V 10	40	3.7	4.3	1.4	3.8	1.2

Plate XI. Mandibular Incisors and Pre-Molars of Group II Animal
Note: Lengthened incisor curling backwards and irregular pre-molars.

Plate XII. Mandibular Incisor, Molars and Pre-Molars of Group IV Animal
Note: Shortened incisors with lesser curvature and regular molars and pre-molars.

Plate XIII. Mandible of Group II Animal
Note: Irregular and overgrown pre-molar in background and general irregular pre-molar and molar distribution.

Plate XIV. Mandible of Group IV Animal
Note: Regular pre-molars and molar distribution.

fluoride or greater resulted in increased length of mandibular incisors (Plates XI and XIII). The length of mandibular incisor did not appear to be proportional to fluoride intake, but appeared only to be stimulated to greater rates of growth when levels of fluoride in water were in excess of 20 ppm. Again Group II Animal II had shown the greatest response to the fluoride treatment.

2. Rabbits

The Polish Rabbit appears to be less susceptible than the chinchilla to fluoride administration as illustrated by Table XIX. From this chart, one observes that very little variation in length of mandibular or maxillary incisors resulted from exposure of experimental rabbits to three different levels of fluoridated water.

B. Spacing Between Incisors

1. Chinchillas

Since no recognizable gaps existed between either sets of incisors of chinchillas, data was not obtained.

2. Rabbits

Data from Table XX indicates that the space between maxillary incisors was decreased as level of fluoride administration in water was increased. No such trend was observed with the mandibular incisors. Because of the small sample size, these observations were not considered to be significant.

C. Angle of Tooth Eruption

1. Chinchillas

In general, the greater angle of the mandibular incisors was decreased as fluoride levels of water were increased (Table XXI, Plate

TABLE XIX. Tooth Measurements Versus Fluoride Treatment in Polish Rabbits

Animal	Level of Fluoride in Water (ppm)	Diameter of Skull as Measured by Circumference at Orbital sinus (cm)	Length Upper Incisor (mm)	Length Upper Incisor/Unit Skull Dimension (mm/cm)	Length Lower Incisor (mm)	Length Lower Incisor/Unit Skull Dimension (mm/cm)
III 1	0	4.2	4.2	1.0	4.2	1.0
I 1	20	4.0	4.4	1.1	3.3	.8
I 3	20	4.3	3.8	.9	3.2	.7
I 6	20	4.0	3.1	.8	6.5	1.6
I 7	20	3.7	4.2	1.1	3.1	.8
V 4	40	4.1	3.5	.9	4.6	1.1
V 5	40	3.6	2.9	.8	3.8	1.0
V 6	40	4.0	3.3	.8	4.2	1.1

TABLE XX. Measurement of Space Between Lower Incisors of Polish Rabbits Exposed to Various Levels of Fluoride Administered via Drinking Water

Animal Group	Animal	Level of Fluoride in Water (ppm)	Space Between Maxillary Incisors (mm) Value	Average	Median
III	1	1	3.4	3.4	3.4
I	1	20	5.8		
I	3	20	3.8	3.5	2.9±.3
I	6	20	4.3		
I	7	20	-		
V	4	40	1.8		
V	5	40	2.9	2.5	2.4±.5
V	6	40	2.9		

TABLE XXI. Angle of Tooth Eruption Versus Fluoride Administered in Chinchillas

Animal	Level of Fluoride Administered Via Water (ppm)	Lower Incisor Greater Angle (degrees)	Lower Incisor Lesser Angle (degrees)	Upper Incisor Angle (degrees)
IV 1	0	145	121	65
IV 2	0	141	123	58
IV 4	0	139	110	48
III 2	1	131	115	66
III 3	1	149	121	53
III 4	1	141	125	60
I 3	20	129	115	62
I 5	20	132	121	53
II 2	10	136	115	40
II 3	10	141	132	61
V 3	40	154	114	50
V 4	40	145	125	46
V 6	40	129	113	44
V 7	40	155	117	45
V 9	40	125	117	52
V 10	40	143	128	62

XI and Plate XII)to 10 ppm fluoride or greater. Essentially, this meant that teeth grew more perpendicular to the jaw as levels of water fluoride were increased to 10 ppm fluoride or greater. The lesser angle of the mandibular incisors (an indicator of the caudal curving of the teeth) also became decreased (Table XXI, Plates XI and XII)as fluoride levels of water were increased in excess of 10 ppm fluoride. Essentially, as fluoride administration via the water was increased to 10 ppm fluoride or greater, a greater proportion of experimental animals developed mandibular incisors that grew upwards and caudal. Table XXI also demonstrated that when fluoride levels of water were 20 ppm or greater, the maxillary incisors tended to grow caudal into the buccal cavity.

2. Rabbits

Although certain rabbits (Group I Animal 6, Group I Animal 7, and Group V Animal 5) exposed to higher levels of fluoride in the water developed a trend towards the caudal growth of the mandibular incisors (as measured by the lesser angle of mandibular incisors of Table XXII), no direct correlation between angle of mandibular incisor and fluoride level could be obtained. The maxillary incisors, however, tended to develop a more caudal growth as fluoride levels of water were increased.

D. Tooth Quality

1. Chinchillas

From information from Table XXIII, only two experimental chinchillas had any major incisor abnormalities--Group I Animal 1 which had one maxillary incisor missing and Group II Animal 2 which had excessively long and slender mandibular incisors that were severely pitted. All molars and pre-molars of Group II Animal 2 displayed altered lengths

TABLE XXII. Angle of Tooth Eruption and Tooth Quality Versus Fluoride Administered via the Water in Polish Rabbits

Animal	Level of Fluoride in Water (ppm)	Lower Incisor-Greater Angle (degrees)	Lower Incisor-Lesser Angle (degrees)	Upper Incisor Angle (degrees)	Comments
III 1	0	145	125	85	
I 11	20	140	127	71	
I 3	20	145	125	84	
I 6	20	145	111	72	Long incisors, pre-molars of unequal proportions
I 7	20	140	110	79	
V 4	40	137	125	75	altered proportioned molar and pre-molars
V 5	40	143	115	85	altered proportioned molar and pre-molars
V 6	40	163	133	78	altered proportioned molar and pre-molars

TABLE XXIII. Tooth Quality Versus Fluoride Administered in Chinchillas

Animal	Level of Fluoride Administered Via Water (ppm)	Color of Teeth (Deep Orange-Clear) (6 - 1)	Mottled	Comments
IV 1	0	5	no	
IV 2	0	5	no	
IV 4	0	5	no	
III 2	1	4	yes	
III 3	1	4	yes	
III 4	1	3	yes	
I 3	20	6	no	only 1 incisor present
I 5	20	3	no	
II 2	10	3	yes	incisors long and slender, pitted, rough, all molars and pre-molars out of proportion
II 3	10	6	no	
V 3	40	5	no	
V 4	40	2	very	
V 6	40	5	yes	
V 7	40	4	yes	
V 9	40	5	no	
V 10	40	4	yes	

and proportions. Plates XI and XIII (Group II Animal 2) and Plates XII and XIV (Group IV Animal I) contrast extremes of the two types of teeth observed. Plates XII and XIV demonstrate the normal tooth arrangement typical of Group IV chinchillas. Plates XI and XIII demonstrate the type of molar and pre-molar abnormalities typical of susceptible animals exposed to high fluoride diets. Contrast the uniformly sized and shaped teeth of Plate XIV to the abnormal proportions and positions of teeth seen in Plates XI and XIII. Note the pre-molar in the background of Plate XIII that is more than double the length of any other pre-molar.

2. Rabbits

No incisor abnormalities were observed in the experimental rabbits but the molars and pre-molars of rabbits appear to be extremely susceptible to elevated fluoride intake. Table XXII demonstrates that all animals, exposed to water containing 40 ppm fluoride, developed molars and pre-molars of irregular size and shape.

E. Tooth Color

1. Chinchillas

Although the normal orange pigmentation of chinchillas' incisors tended to disappear (Table XXIII) as fluoride intake levels were increased, no significantly valid variations in tooth color were observed. Even though white striations occurred in the incisors of many experimental chinchillas such "mottling" was not found to be proportional to fluoride intake.

2. Rabbits

Since rabbits did not possess incisors with orange pigmentation, color variations were not observed.

DISCUSSION AND CONCLUSIONS

It has been reported by numerous workers (Eager, 1901; McCollum et al., 1925; Lipkins et al., 1959; Takamori, 1964; Simons, 1965) that an excessive fluoride intake may result in a wide variety of acute and subacute physiological abberations. This thesis has confirmed the fact that some abnormalities occurred as a result of the addition of up to 40 ppm soluble fluoride to the drinking water of rabbits and chinchillas.

No significant variations in final body weight or body weight gain were observed in chinchillas exposed to an excessive (up to 40 ppm) fluoride intake. These observations resemble those results for rats treated at similar fluoride levels reported by Auskaps and Shaw (1955).

The data obtained from the metabolic trials performed on chinchillas had several interesting features. The first observation was that as the fluoride levels of the drinking water were increased, water consumption increased, resulting in an increased total fluoride intake of the experimental animals. From the data obtained from the studies on water consumption (Table VII) in chinchillas, it was obvious that once fluoride levels of the water reached the level of 20 ppm, water consumption became greatly increased. Since no visual evidence was obtained to indicate that this excessive water consumption was due to increased water wastage by the chinchilla, it was assumed that the high fluoride content of drinking water resulted in a stimulated thirst of the chinchillas. The data also indicated that seasonal variations could exist in rate of water consumption. Since abnormal variations in temperature, light or

humidity did not occur, this seasonal variation in water consumption must have been a result of parameters not controlled or observed (such as atmospheric pressure).

The data from the metabolic trials demonstrated that a positive fluoride balance (apparent storage of fluoride) was produced in each of the chinchillas tested. The level of this fluoride balance was observed to be directly proportional to the level of fluoride (in drinking water) administered to the research animal. It was demonstrated that the amount of fluoride excreted in the feces was a direct function of the amount of fluoride ingested as feed. From the metabolic trials performed on chinchillas receiving no fluoride via the water supply, it was shown that approximately one-third of the fluoride administered as feed remained in the chinchillas body.

The third finding of the metabolic trials was that despite extreme variations in the level of fluoride administration, urinary fluoride excretion levels remained relatively constant. From this, it appears that chinchillas have an extremely low renal clearance for fluoride. This finding is opposed to much of the current thinking concerning fluoride metabolism in other animals. Hodge (1961) demonstrated that the fluoride absorbed by rats was excreted rapidly and almost entirely in urine. Even when Smith et al. (1955) induced a high grade tubular injury in the kidneys of rabbits by uranium administration, normal fluoride-urine concentrations were observed. The thesis findings indicate that individuals (such as commercial chinchilla breeders), supplying their breeding colony with fluoridated drinking water at levels of 20 ppm fluoride or greater, could experience complications

with respect to fluoride accumulation.

The data on clinical blood assays performed on chinchillas and rabbits verified McClure and Kornlay's (1947) conclusions that increased fluoride intake resulted in little or no variations in hematocrit. Although variations in certain blood cell types were demonstrated in the research on rabbits, the sample size and disease syndrome present made these results unreliable.

Although certain rabbits exposed to high fluoride water supplies had kidney and liver fluoride levels higher than control animals, these tissue fluoride levels all fell into the normal fluoride levels established for these tissues by Simons (1965).

From the data, one would conclude that breaking strength of chinchilla bones was increased when fluoride intake via the water increased. Had the experimental sample size been larger, this trend may have been more obvious. This increased bone strength was not a result of alterations in levels of calcium or phosphorus since these levels remained relatively constant, as did the calcium to phosphorus ratio. The increased bone strength could have resulted from increased levels of fluorapatite in the bone.

Although a mandibular incisor malocclusion or overgrowth, similar to that described by McCollum et al. (1925), was not reproduced in chinchillas or rabbits, an overall increase in mandibular incisor length was observed in chinchillas exposed to drinking water fluoride levels in excess of 20 ppm. Coinciding with this incisor growth was the tendency of the mandibular incisors of chinchillas to have decreased greater and lesser angles of tooth eruption as levels of fluoride were

increased beyond 20 ppm. This essentially means that chinchillas exposed to fluoride levels equal to or greater than 20 ppm (in drinking water) had incisors longer, more erect and tending to grow backwards into the buccal cavity. Since Polish Rabbits had mandibular incisors that did not follow this trend, one could conclude that the chinchillas' incisors (the osteoblasts) were more susceptible to malocclusion resulting from excessive fluoride intake than were the rabbits'. Since both the rabbits and chinchillas, given fluoride in the water at levels of 20 ppm or greater, had malformed molars and pre-molars, one could conclude that the molars and pre-molars of both species of animals were very susceptible to fluoride addition. Because the pigmentation in the teeth of chinchillas remained constant despite variations in fluoride treatment, one could conclude the mechanisms for incisor pigmentation were independent of the presence of fluoride.

From the discussion reported above, it becomes apparent that no obvious physiological abnormalities occurred in rabbits or chinchillas exposed to experimental drinking water containing less than 20 ppm fluorice (45.2 ppm fluoride in the total diet). Once fluoride levels of drinking water reached the threshold level of 20 ppm, numerous body changes became apparent in several experimental rabbits and chinchillas. Many of these abnormalities did not occur consistently throughout each experimental group. As a result, biological variation and small sample size played a major role in the analysis of much of the experimental data. Had sample sizes of each experimental group been larger, many of the observations and conclusions could have been considered more reliable. Midway through the experimentation on rabbits, an acute protozoan disease

syndrome, Nosematosis, presented itself. It is difficult to conclude whether this disease syndrome was the result of a subacute infection irritated to produce an acute disease syndrome by experimental stress or whether it was introduced to the rabbit colony from an outside source. Regardless of its cause, validity of much of the data pertaining to fluoride intake in rabbits must be questioned because of the effects of this disease.

From the data, observations and discussion included in this thesis, the work can be summarized as follows:

1) No variations in total body weight or body weight gain in chinchillas resulted from increased fluoride intake.
2) An increase in water consumption by chinchillas resulted from the increased fluoride intake. Water consumption also appeared to exhibit seasonal fluctuations.
3) Although the chinchillas fluoride intake and absorption increased as fluoride levels of water increased, the renal system did not appear to have an efficient means of eliminating this fluoride from the body. As a result, high apparent storage of fluoride (fluoride balance) was demonstrated.
4) No variations in hematocrit or tissue fluoride levels were apparent in rabbits or chinchillas exposed to increased fluoride intake.
5) Increased mandibular incisor length and decreased mandibular incisor eruption angles in chinchillas resulted from increased fluoride intake.
6) Increased molar and pre-molar malocclusions in rabbits and chinchillas resulted from increased fluoride intake.
7) No variations in tooth pigmentation in chinchillas resulted from increased fluoride intake.

Because of the experimental design, it was impossible to ascertain whether the abberations observed resulted from the effects of the presence of high levels of the fluoride ion or from a general systemic poisoning resulting from excessive fluoride intake. It is apparent that fluoride levels of water under 20 ppm are safe to the normal health of both rabbits and chinchillas. Fluoride levels of municipal water supplies, are, therefore, safe for the production of these experimental animals.

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